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JOURNAL

OF THE

NEW-YORK MICROSCOPICAL SOCIETY

VOL. X. JANUARY, 1894. No. 1.

ON UNIFORMLY STAINED COVER-PREPARATIONS OF MICRO-ORGANISMS, FREE FROM DISTORTION.

BY ALEXIS A. JULIEN, PH.D.

(*Read November 3d*, 1893.)

The morphological differences between the kinds of bacteria will be probably found as distinct as in all other micro-organisms. Recent investigations have begun to emphasize their specific peculiarities in internal structure and enclosures and in their outer organs of motion, and the value of these in diagnosis of species. A common impression to the contrary, long and often expressed among bacteriologists, is certainly based, I think, on unsatisfactory results which have been naturally derived from some present easy methods of preparation of material, the use of lenses of easy-working distance but narrow aperture, and easy but ineffective methods of manipulation. The higher needs of modern bacteriology surely call for methods of patient and painstaking treatment analogous to those long used on histological preparations. There are serious objections to the process, now in almost universal use, for the mounting of pure growths of bacteria and micro-organisms, and enjoined in most text books[1]—the smearing of covers with droplets of the growth under the edge of a slide drawn across the surface, or by rubbing the droplet

[1] E. M. Crookshank, "Manual of Bacteriology," 3d Ed. (1890), 65. G. M. Sternberg, "Manual of Bacteriology" (1892), 26, 27.

between two covers, afterward drawn apart; the completion of the drying of the film on the cover by passing it to and fro through a flame; and the staining in simple solutions of anilin colors in water, anilin-water, or alcohol. No worker should any longer waste his time and labor on such a process, with its known miserable results in imperfect mounts and the unsatisfactory conclusions they must yield.

1. The material is spread irregularly. On account of its ordinary glutinous character and excessive richness in forms, the film on most bacteria mounts is either rendered too dense, crowded, and opaque, or, at the other extreme, is represented by a few scanty wisps or streaks, for which a tedious search must be made all over the cover.

2. The true structure and grouping of the bacteria are disturbed or destroyed. In the rough process of smearing, the delicate attachment of the elements of bacterial filaments and of groups of cocci, often loosely aggregated, is rudely broken up. In place of bacterial chains, the student often obtains a film, partly or wholly consisting of desiccated and widely scattered bacilli; in place of streptococci, groups of four, grape-like bunches and cubical packets, he finds solitary cocci and perhaps a few lonely diplococci; and, very likely, his original spirilla and even vibrios have been nearly all rent apart into isolated curved bacilli; there is now a question whether some of the so-called spirochæte may not be but the paddles torn away from the bodies of roughly handled hæmatozoa.

As the discrimination of bacterial species, still very difficult, depends partly, often largely, on recognition of original forms and grouping, the present destructive and clumsy process of smearing has often long delayed the detection of important facts (*e.g.*, the spirillum form of the organism connected with Asiatic cholera) and ought to be entirely rejected. For these reasons several writers have recently recommended the previous dilution of the original bacterial growth with sterilized water upon the cover, or before its application to the cover. In fact, we have had one truthful process, in preservation of bacterial forms, that of cover-impressions from the surface of solid and liquid media; but these have been of limited and often difficult application.

3. Further distortions of form occur during the processes of

drying of the film. The elements of groups are falsely disturbed, even with those lucky organisms which have escaped injury during the preceding rough treatment. These may be due to contraction during drying, and it may affect even forms and groups whose shapes allow them to be pressed down upon a plane surface without distortion, such as rods, cocci, chains, and merismopedia. The bacteriologist is familiar with the faulted lines and short offsets which signify irregular shrinking during overhasty drying of these delicate watery organisms, and with ghostly lines and spots showing where they have drawn aside or often split entirely away from the cover. But with those forms which must obviously become distorted when flattened upon a plane surface, such as many vibrios and all spirilla, spirochæte, staphylococcus bunches, and sarcina packets, any process of drying upon covers must be objectionable on account of this distortion. Still further, during the drying of ciliated forms in active motion, even at the natural temperature of the laboratory, a serious cause of deformation arises from the writhing toward the end of the drying, especially in forms which become cemented at one end to the cover, while the remainder continues twisting and wriggling into strange, often fractured shapes. These contortions and dislocations can be actually watched during the struggles of the entrapped micro-organisms, and are abundantly displayed on any film of dried spiral bacteria and in numerous published photomicrographs; spirilla are shown twisted into a curve, spirochæte actually bent at right angles, etc. A recent example of such distortion is shown in the photomicrograph of *Sp. volutans* by Dr. R. L. Maddox, accompanying his interesting paper in the *Journal* of the Royal Microscopical Society for December, 1893; he had already shown the true form in his previous paper in the *International Journal of Microscopy and Natural Science*, iii. (1893), page 233.

The needed precautions, in my opinion, are, first, that all micro-organisms, including bacteria, should be suddenly killed and fixed before evaporation of the film or any other process of mounting, especially those of active movement; secondly, that such evaporation should be carried on very slowly and at a low temperature, especially with curved and thick organisms. One purpose of the evaporation, especially the later heating in or over

a flame, has been the coagulation of albuminoids in the liquid of the drop, to prevent general coloration of the field during staining. But this is the common cause of resistance to staining by delicate inner details and the cilia, while it can be well effected by immersion in a proper fixative. A second purpose of this excessive drying has been the supposed necessity to insure adherence of the film to the cover. But I have found that when a film is well dried at low temperature, as explained beyond, no peeling away or loss afterward occurs.

4. The staining is irregular and unsatisfactory. In most bacteria mounts, as met everywhere on exhibition, these forms are greatly overstained and look all alike, presenting mere silhouettes of the contours, with few or no traces of inner structure and with cilia entirely invisible. For a long time it has seemed to be the acme of effort to render these microbes visible at all. This has been followed by special efforts to effect satisfactory staining of spores or of cilia, in separate preparations. Nor is it strange that many bacteriologists have concluded that these silhouettes show the prevalence of such a sameness of form among the kinds of bacteria that the main dependence, for discrimination, must be placed on other characteristics of a physiological nature, modes of growth on different media, etc.

But these opaque caricatures of the bacteria are as unworthy of modern science and as unnecessary as similar misrepresentations of the infusoria or other micro-organisms would be considered. The improvement of modern lenses of wide aperture, and increasing facility in their manipulation, are gradually leading as well to more successful approach toward the preparation of an ideal bacteria mount. This should of course present the groups or chains dispersed over the whole cover, with natural arrangement undistorted and unbroken, with normal mode of interconnection, and with constituent rods or cocci just sufficiently stained to display both inner structure and external organs. To this end the anilin stains are commonly misused, being greedily and excessively absorbed by the plasm within the cells, while cellulose, callose, etc., remain unaffected. With a dried bacteria film, therefore, the preliminary use of some mordant is indispensable for staining, to restore the absorbence in all parts of the organism.

However, it is probable that the drying and heating of the bac-

teria film, perhaps to any degree, must cause such contraction of the sensitive plasm within the cells and of that connected with the cilia as to involve just this known resistance to coloration. In several recent investigations there has been a return to simple processes by which, without drying or heating, the living bacteria have been successfully stained throughout, including the cilia ; for example, that of N. Sjöbring,[1] who, in studies of their structure and nuclei, used nitric acid as fixative, stained with carbol-methylen-blue or carbol-magenta-red, decolorized with nitric acid, and examined in glycerin and water ; that of Straus,[2] who merely added diluted Ziehl's solution to a loopful of bouillon culture of several kinds of spirilla ; that of Klein,[3] who used, on the living spirillum of Asiatic cholera, a mixture of equal parts of absolute alcohol and a solution of gentian-violet in anilin water, during five to ten minutes ; and that of R. L. Maddox,[4] who used saturated solution of tannic acid as fixative, and then added a saturated solution of iron sulphate, containing about two per cent of citric acid, for the staining of spirilla. Not only, therefore, is it certain that the process of drying and overheating bacteria on thin covers long delayed the discovery of cilia and other details and is yet impeding their investigation, but it is probably responsible for some of the varying conclusions of modern workers concerning distribution of chromatin, septa, nuclei, spores, and cilia through artificial and false structures developed by contraction.

To recapitulate, the new steps suggested by these considerations, when it is thought desirable to prepare a dried bacteria film, are as follows : preliminary dilution of a droplet of the bacterial growth in sterilized distilled water ; killing and fixation of the bacteria, in a drop upon a thin cover, by addition of suitable fixative ; slow evaporation at a low temperature ; and immersion in a mordant before staining.

As to the selection of fixative, I have tried weak aqueous solutions of the following reagents, in succession or in comparative series, during the recent preparation of two hundred and thirty mounts of several species of ciliated bacteria, spirilla of Beggiatoa, spirilla undula, etc. As the fixative cannot be removed from

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[1] Centralbl. f. Bakt. u. Par., xi. (1892), 65.
[2] Idem, xiv. (1893), 257.
[3] Idem, xiv. (1893), 618.
[4] Jour. Roy. Mic. Soc. (1893), 718.

such minute bodies as the bacteria, before evaporation, but must be evaporated with the drop on the cover, the results of such concentration of each fixative must be considered in determining its value. The following were found to be efficient in killing instantaneously and as fixatives, but were all objectionable in that their intermixture with the bacteria in the form of crystalline tufts or flakes, by concentration during the evaporation of the drop, seemed to lessen the adherence of the film, which separated in spots during subsequent processes of the preparation: viz., sodium chloride, iron sulphate, iodine in solution of potassium iodide, chloral hydrate, quinine sulphate, morphia sulphate, hydroxylamin, Loeffler's mordant, and fuchsin solution. Of these sodium chloride was the simplest, and often quite efficient. Osmic acid, picric acid, ether, and chloroform were more satisfactory, but especially absolute alcohol, though this last reagent was objectionable on account of the violent currents produced on its addition in a droplet. The best results were obtained with hot water, tannin, chromic acid (in water solution, diluted until colorless by daylight), and hydrogen peroxide.

As to the staining process, that of Loeffler, with two solutions, mordant and colorant, though specially devised for the staining of cilia, appears well adapted for the staining of the entire bacterium. However, in place of the unstable salt, iron sulphate, recommended in his formula, it seems better to use, as suggested by L. Luksch,[1] a cold saturated solution of ferric acetate, with addition of a few drops of acetic acid. The further addition to the mordant—on which Loeffler lays so much stress—of a few drops of solution of sodium hydrate or of hydrochloric acid, according to the alkaline or acid reactions of the natural products of growth of the bacterium which is to be stained, has been found unnecessary by M. Nicolle and V. Morax.[2] With this my own experience coincides; no actual change of reaction is produced in the mordant, nor any improvement in the results; the variable and uncertain staining of cilia, often observed, seems to be dependent on quite other conditions, probably connected with the anterior drying of the film. The two solutions as simplified

[1] Centralbl. f. Bakt. u. Par., xii. (1892), 430.
[2] Ann. de l'Inst. Past., vii. (1893), 554.

by Loeffler in his second method,[1] and modified as already suggested, are now made in the laboratory of Micro-Biology at Columbia College as follows :

Mordant.—To 10 c.c. of solution of tannin (20 per cent in water) add commercial solution of iron acetate, drop by drop (about 5 c.c.), until a violet-black is produced without precipitate. Then add 5 to 10 drops of acetic acid and 4 c.c. of solution of carbolic acid (12 per cent in water), and filter. With ordinary protection from the air, the solution is stable for a long time.

Loeffler also adds 1 c.c. of fuchsin solution to the mordant, and so also Nicolle and Morax, with the special purpose to insure staining of the cilia. As my own object is the more general one of effecting a uniform and harmonious coloration of the entire bacterium, I find the results under better control by confining the anilin color to the colorant proper.

Colorant.—To 100 c.c. of anilin water add solution of sodium hydrate (1 per cent in water), drop by drop (about 5 c.c.), until a neutral reaction is obtained with test papers. Then add $\frac{1}{2}$ gramme of fuchsin and shake until solution; but filter every time just before using. From time to time, as the solution loses color by decomposition, add a little more fuchsin and shake up before filtering.

In the original process, with exclusive object of staining cilia, Loeffler uses 4 grammes of fuchsin, and Nicolle and Morax, Ziehl's fuchsin—both solutions being saturated with stain and opaque. On films prepared by my method, fixed and dried with little or no heat, the results appear more satisfactory with an alkaline solution like that of Loeffler, though containing but one-eighth of the amount of stain he prescribes, brightly colored but transparent.

A supposed improvement of Loeffler's process has been advanced by A. P. Brown,[2] who substitutes as mordant a cold alcohol solution of tannin and anilin oil, in which the dried bacteria film is to be immersed for two to five hours or over night. This method also I have tried for about a month, and, although it often yields excellent results, it has appeared to be less certain and uniform than the hot mordant recommended by Loeffler.

[1] Jour. de Micrographie, xv. (1891), 269.

[2] The Observer, iii. (1892), 293, and Jour. Roy. M'c. Soc. (1893), 268.

The latter has also the great advantage of speed, as the mordanting is effected in less than a minute.

Both Loeffler, and Nicolle and Morax, have advised the repetition of application of the mordant two or three times in some cases. In this I have found no apparent gain; the entire action of the hot mordant on these minute bodies seems to be completed during a single brief immersion.

In all these methods much loss of time would be incurred from treating the covers, as advised, one by one, with drops of mordant and of colorant over a flame. I have elsewhere[1] already indicated the far easier, quicker, and more convenient staining carried on in a wire spring holding a dozen or more covers, with film downward, in the hot mordant or hot stain in a small flask. The dried coloring and overstaining may be thus obviated, for which Loeffler prescribes washing in absolute alcohol.

We may now consider the application of these methods to various micro-organisms, availing ourselves of useful suggestions from the writers referred to.

Preparations of Bacteria.—In making mounts from pure cultures the following process is now used in our laboratory. After drying of the film it ordinarily requires only ten to fifteen minutes for the staining and complete mounting of the preparation. In three or four drops of sterilized water, in a flamed watchglass, stir very gently a particle or droplet of the pure bacteria culture (taken preferably from the surface of a growth on potato or agar) on a platinum wire loop or öse until a slight cloudiness is produced; then take it all up at once within a sterilized drop-tube with rubber bulb. Lay a series of thoroughly cleaned thin covers (treated in Seiler's solution and washed in distilled water and pure alcohol) on the bottom of a shallow two-inch Petri dish. Apply to each, and spread very gently, a drop of the diluted culture, and immediately add to the centre of the drop a droplet of the selected fixative (say, tannic acid or chromic acid solution). If a sparser distribution of the bacteria is desired, allow the drop to settle a few minutes, take up and incline each cover, and remove excess from lower edge. Allow to dry, with protection from dust, at the natural temperature of the room, or

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[1] "Suggestions in Microscopical Technique," this JOURNAL, ix. (1893), 26.

over concentrated sulphuric acid within a desiccator, or, still better, in vacuo over sulphuric acid, under an air-pump.

When thoroughly dry, insert all the covers in the brass wire coil, with film surfaces all directed toward its lower end, and with arranged and noted succession for identification, if different kinds of bacteria are included in the series. With some species it appears desirable to hold the coil a few seconds in a current of warm air to complete the drying.

Dip the coil for a moment in sterilized water, to dissolve away excess of fixative, remove the water drops by touching absorbent paper, and again dry the covers.

Hang the coil for one-half to one minute in the flask of mordant, heated nearly to boiling. Remove the coil and take up excess of mordant by touching absorbent paper ; wash by swinging gently, for about five seconds, in a vessel of distilled water, then in solution of acetic acid (20 per cent), again in water, and remove water drops by touching absorbent paper.

Without drying, hang the coil in the flask of hot colorant for five to sixty seconds, according to depth of staining desired and the readiness of absorbence of the species, determined by trial. Remove the coil, take up excess of stain by touching absorbent paper, and wash as before in another vessel of distilled water.

Remove the covers from the coil, and lay, film downward, on a soft filter paper. Dry by pressing gently under another filter. Clean the upper face of each cover by rubbing with corner of rag moistened with alcohol, keeping the cover stationary to avoid rubbing away the underlying film. Lay upon a glass slide, remove to stage of microscope, and examine and select the covers preferable for mounting.

Mount in hardened balsam, or in a saturated solution of potassium acetate within a very shallow spun cell of balsam-paraffin, King's cement, or Hollis glue. In cases, delicate details of structure may be best shown by mounting in air—*e.g.*, over a filmy ring of paraffin.

The fugitive character of staining effected by anilin colors has recently led E. Van Ermengem to devise the following process[1] of staining the cilia of bacteria, founded on the reduction of silver from a solution of its nitrate.

[1] Ann. de Micrographie, v. (1893), 394.

The well-dried bacteria films are immersed half an hour (or, if need be, five minutes at 60° C.) in the

Fixing Bath.

Osmic acid (2-per-cent solution)	1 part.
Tannin (10- to 25-per-cent solution)	2 parts.

They are then washed with utmost care in water and in alcohol, and placed a few seconds in the

Sensitizing Bath.

Silver nitrate, a very weak solution (0.5 to 0.25 per cent).

Without washing, they are next subjected to the action of the

Reducing Bath.

Gallic acid	5 parts.
Tannin	3 "
Melted sodium acetate	10 "
Distilled water	350 "

After a few moments they are returned to the silver bath, removed when the bath begins to blacken, washed thoroughly, dried, and mounted in balsam.

I have made trial, with some success, of this promising method, in reference to the more general object of the present paper. So far I find an objectionable tendency to blackening and overstaining of the whole preparation, which I think may be obviated by reducing the strength of the baths.

The successful results already obtained in direct staining of living bacteria by simple processes, like those of Sjöbring, Straus, Klein, and Maddox, warrant the prophecy that some such process, with or without application of mordant, as tested on each kind, will yet come into general use when the utmost perfection is desired in a preparation. In other cases such staining of living bacteria may well precede their drying on covers in the ordinary way, when the containing liquid is sufficiently free from substances inclined to absorb the stain.

Preparations of Spirilla.—In regard to spirochæte, spirillum, and vibrio forms, the preliminary process of killing with a fixative, before evaporation of the film, was found to have materially

reduced the number of distorted spirals, apparently all that would have been produced by writhing movements of organisms partially adhering to the cover. Only those remained that were inseparably connected with the process of flattening down spiral forms upon a plane surface. To eliminate this last source of deformation the following process has been devised, which requires some care and patient manipulation, but yields the spirilla in perfect preservation. There is, of course, greater difficulty of studying a spiral under a high magnifying power, and a troublesome tendency of these spiral forms to intertwist in bunches during concentration.

Put a sufficient quantity, say 20 c.c., of the liquid containing the living bacteria in a conical sherry glass or in a urinary deposit tube. Add a little fixative, say 5 c.c. of tannic acid or chromic acid solution, mix by shaking gently, and allow to settle for at least half an hour. Draw off the supernatant liquid, with utmost care not to disturb the light invisible deposit, and wash by a change of sterilized water, allowing to settle as before. Draw off the liquid and transfer the deposit to a number of slides with very shallow cells. Add to each a drop of cold colorant, remove excess by slips of absorbent paper, and wash by changes of sterilized water, removing each by absorbent paper. Then add saturated solution of potassium acetate, cover, and seal.

Preparations of Amœboid Organisms.—The difficulty of preparing permanent mounts of amœbæ, foraminifera, etc., is shown by the rarity of such preparations in all collections and exhibitions. The long-accepted but unfounded conviction as to the constitution of such forms of but slightly differentiated and therefore unstable protoplasm has probably led to discouragement of much effort in this direction.

Certes has advised the following process[1] for preparation of mounts of amœbæ. To 30 c.c. of the water containing the living amœbæ about 1 c.c. of osmic acid solution (1 per cent) is added. After settling a few hours, the deposit is washed, concentrated, stained, and mounted in distilled water containing a trace of osmic acid.

I have not succeeded by this method, partly perhaps on account of the loss of amœbæ in the confusedly heaped-up mass of asso-

[1] R. Gérard, Traité pratique de Micrographie (1887), 383.

ciated algæ and infusorians, the bending and distortion of the flexible pseudopods, and the obscuring effect of the common black deposit of reduced osmic oxide. My discovery, on examination of preparations of bacteria by the method already explained, that accidentally I had also successfully mounted some associated amœboid forms, has resulted in the following process. It has so far been found satisfactory with *Amœba radiosa*, small forms of *A. princeps*, *Arcella acuminata*, *Actinophrys sol*, and some amœboid forms. From present want of proper material I have been unable to determine what modifications may be needed with the larger forms of amœbæ, and of other rhizopods with bulky gelatinous material, to prevent distortion by contraction during the drying of the film.

The thoroughly cleaned thin covers are laid on the bottom of a small Petri dish or similar shallow tray ; to each a large drop of the water containing the living organisms is added, and then set aside in a "moist chamber," in a quiet, dark, but warm place, for fifteen to sixty minutes. The object is to allow sufficient time for the rhizopods to recover from alarm and to creep about, projecting their pseudopods in a natural way, and yet to prevent any evaporation of the drops. A simple method is to fill a common finger bowl half full of warm water (at 30° to 35° C.), to set the shallow dish or tray floating upon the surface of the water, and to cover the whole from the light with a large wet towel folded several times. The vessel is then gently uncovered, without jar, and to each drop a droplet of fixative is added quickly by touching from a drop-tube, but not by dropping, as that would tend to cause tremor in the drop. As fixatives, hydrogen peroxide, absolute alcohol, chromic acid solution, and, perhaps best of all, osmic acid solution (1 per cent), were used. The tray is then removed from the "moist chamber," the drops are allowed to evaporate, as in the process for preparations of bacteria, and the films treated as before explained.

Also, to a drop of water containing rhizopods under observation, on the stage of a microscope (an inverted stand is preferable), under a half-inch objective, the droplet of fixative may be directly applied at the proper moment of the projection of the pseudopods, and the evaporation, etc., then carried on as already described.

Preparations of Infusorians.—Excellent methods have been devised for preparations of these organisms by Certes, Künstler,[1] Fabre-Domergne,[2] and others. My method, by evaporation, is perhaps only applicable to the smaller forms. With the larger, hitherto, in my experiments, the dried organisms become injured by bursting and outflow, or by irregular contraction, the contour of the sac even parting often from the cilia. With smaller forms the process specified for bacteria may be followed, with use of the special fixative found by trial best suited to each species—*e.g.*, concentrated solution of osmic acid (Künstler), boiling solution of tannic acid, 3 per cent (C. O. Sonntag),[3] equal parts of osmic acid solution (1 per cent) and acetic acid (20 per cent), Kleinenberg's solution, alcohol, chromic acid, picric acid, etc. The dried minute infusorians, like the smaller rhizopods thus prepared, seem as well adapted for study as the bacteria in a dried film, as they display the cilia, sac, vacuoles, nuclei, enclosed food particles, etc., in a satisfactory state of preservation, with no visible distortion.

In place of drying the larger infusorians, the following method has been found useful. They are first narcotized in the drop of water, under observation with a low power, after the method of E. A. Schultze,[4] by addition of a neutralized solution of hydroxylamin hydrochloride (0.25 to 1 per cent), or by Rousselet's method[5] for rotifers, by addition of a weak solution of cocain hydrochloride. At the moment the cilia cease to move the organisms are suddenly killed, after Schultze's method, by adding a drop of alcohol, picric acid, or acetic acid; or, after Rousselet's method, by a drop of osmic acid solution (1 per cent), or by a drop of Flemming's mixture (15 parts of chromic acid, 1-per-cent solution; 4 parts of osmic acid, 2-per-cent solution; and 1 part of glacial acetic acid). The organisms are then washed and stained, as described in preparations of spirilla. The use of Loeffler's mordant on the infusorians is found of the highest advantage, as the cilia become readily absorbent of stain to any depth of coloration desired. A serious difficulty is found in the

[1] Jour. de Micrographie, x. (1886), 59.
[2] "Notes techniques sur l'étude des Protozoaires," Ann. de Micrographie, ii. (1889), 545.
[3] Internat. Jour. of Mic. and Nat. Sci., iii. (1893), 306.
[4] This JOURNAL, viii. (1892), 28.
[5] Jour. Quek. Micr. Club, 2, v. (1893), 205.

greedy absorbence of the anilin dye by the protoplasmic sarcode; but the overcoloration may be controlled by proper dilution of the stain. The infusorians are then mounted in balsam, Wickersheimer's fluid, or other preservative, in the usual way.

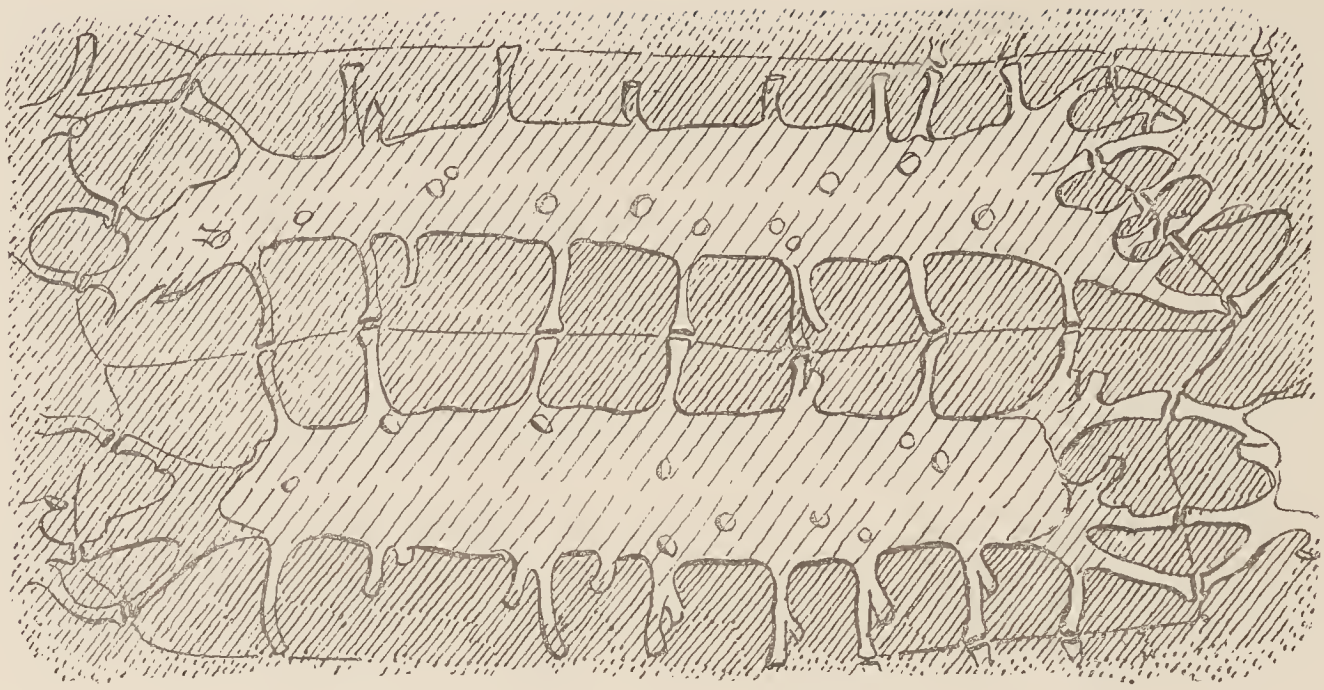
NOTE ON THE STRUCTURE OF THE ENDOSPERM OF PHYTELEPHAS MACROCARPA RUIZ AND PAVON, AND OF SMILACINA RACEMOSA DESF.

BY J. L. ZABRISKIE.

(*Presented December 15th*, 1893.)

1. *Phytelephas macrocarpa.*—The seed of this plant, popularly known as the ivory nut, is an irregular ovate body about one and one-half inches in diameter by two inches in length. When it is cut through the middle transversely and longitudinally the endosperm discloses a "grain," easily seen with a hand lens, resembling in miniature the "grain" of the wood of an exogenous tree. The transverse section shows concentric circles sweeping around the longitudinal axis of the nut. The longitudinal section, through the middle portion of the nut excluding the poles, shows minute longitudinal bands nearly parallel with the axis. Close inspection shows why the poles must be excluded in referring to the bands as parallel with the axis. Rings and bands are found to conform quite accurately with the dark outer surface of the nut, so that when the poles are approached the bands sweep around the latter. It is as if the entire substance of the endosperm were composed of tenuous films or shells of "vegetable ivory," these shells conforming to the outer surface, closely fitting one within the other, and therefore gradually decreasing in size as they approach the axis.

The "grain" is caused by a remarkably uniform arrangement of similar, lengthened cells composing the entire endosperm. But the arrangement is diametrically opposite that which causes the "grain" in the tree. In the tree the cells of the fibre generally overlap each other, and all lie parallel with the axis, and of course parallel with the bark. And a ring or band is caused by the assembling of some of these cells, which are comparatively small



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3

PHYTELEPHAS AND SMILACINA.

in diameter, nearly consolidated by internal deposit, giving a darker hue and firmer texture to that portion of the structure and marking the cessation of the periodical growth of the organism; and so standing strongly contrasted with the immediately superimposed layer of thin-walled, larger cells which mark the periodically resumed activity of growth. In the ivory nut the cells are also somewhat lengthened, but, instead of lying parallel with the axis, they radiate from the axis, and all extend outward toward the dark outer surface and normal to its curve. The cells do not usually overlap each other, but with remarkable uniformity they lie side by side, and meet each other end to end. And the appearance of rings and bands is caused by dense clusters of tubules at the ends of these cells, the members of one cluster accurately meeting the members of another cluster from the ends of adjoining cells, the extremities of the approaching tubules, however, always being separated from each other by the middle lamella between two adjoining cells. These clusters are so dense and are so regularly arranged, on account of the usually regular position of the cells side by side, as well as end to end, that, by contrast with the portion of the cell intermediate between the ends—which intermediate portion, however, is also well furnished with tubules—the transverse section shows attenuated but unbroken, darkened circles sweeping around the axis.

Description of Plate 38.

FIG. 1.—Longitudinal section of two entire cells of the endosperm of *Phytelephas*, with four extremities of adjoining cells, meeting end to end. The cells are split longitudinally and cleared of cell contents. The darker shaded portions represent the upper surface of the section, showing the thickness of the cell walls. The lighter portions represent the semi-cylindrical cavity of the cell, like a minute trough, and also many of the tubules, which lie near the upper surface of the section and pass outwardly to the lamella or boundary of each cell. The small circles represent the openings of such tubules as are passing downward at various angles through the remaining thickness of the section. Many of the tubules at the ends of the cells are cut off at various lengths by the plane of the section.

FIG. 2.—Transverse section of a number of cells of the endosperm of *Phytelephas*, cleared of cell contents and showing the polygonal outlines. The plane of the section passes through the clusters of tubules at one end of the cells. The shaded portions represent the thickened cell walls, and the clear spaces the contracted lumen of this region of the cells.

FIG. 3.—Section of the endosperm of *Smilacina*, with its irregularly globular or ellipsoidal cells, cleared of cell contents. The darkest shaded portions represent those portions of the cell walls which rise directly upward to the upper surface of the section, and which, therefore, exhibit at best advantage the thickening of the cell walls and the abundance of tubules.

The three sketches are all of the same magnification—250 diameters.

Of course in multitudes of instances the end of one cell must meet the ends of two or more cells, to allow for the interposition of radii, as the view advances from axis to periphery—from one ring or film to the next outlying ring or film; but these instances are rare compared with the abounding number of regularly disposed cells in any one section when magnified.

These cells will average about .004 of an inch in diameter by .014 of an inch in length. The cell walls are so thickened by internal deposit that frequently the lumen, or cavity, is left of a diameter of only about one-third of the entire diameter of the cell. It is this deposit which causes the bone-like density of this remarkable vegetable product. The tubules, resulting from the avoidance of this deposit at certain points, are frequently curved and branched, and the clusters of these tubules at the ends of the cells are especially branched and tortuous, as they meet the members of similar clusters not only from the cell which lies directly in advance, but also from those which lie surrounding its polygonal periphery. It is not easy to count them, but the tubules of the cluster at one end of one cell undoubtedly often reach the number of twenty or more.

2. *Smilacina racemosa.*—This plant, of the lily family, and one of the common woodland herbs of our region, reaches a height of one to three feet, with a slender, simple stem, well furnished with oval leaves, and bears small white flowers in a terminal racemose panicle. The berries are pale red, speckled with purple, containing one or two seeds.

A section of these seeds discloses a very homogeneous but confused mass of irregularly globular or ellipsoidal cells, about .003 by .005 of an inch in dimensions, with greatly thickened cell walls, abundantly furnished with tubules, which latter are of large diameter compared with their length. The deposit averages not quite one-half of the thickness of that found in the ivory nut, but it is sufficient to make a near approach to the density of "vegetable ivory." The endosperm of this seed of *Smilacina* is probably the hardest of any recorded instance among the indigenous plants of our region. The seed is so hard that it can be easily driven with one blow of a hammer, and without fracture, flush into the substance of pine wood.

PROCEEDINGS.

Meeting of October 6th, 1893.

The President, Mr. Charles S. Shultz, in the chair.

Twenty-one persons present.

The Recording Secretary read a communication from Prof. W. Goold Levison, requesting the appointment of a committee by this Society to meet with committees of allied societies for the purpose of conference on the adoption of a uniform size for boxes, cases, and cabinets for the display and preservation of microscopical and mineralogical specimens.

Mr. James Walker was appointed as such committee.

OBJECTS EXHIBITED.

1. Capsules of Canada balsam, *in situ*, in the bark of *Abies balsamea* Miller: by Alfred M. Mayer.

2. *Acineta tuberosa.*

3. *Bacillaria paradoxa.*

4. *Megalotrocha albo-flavicans.*

5. *Octocella libertas.*

6. *Urnatella walkerii.*

7. *Cordylophora coronata.*

8. The form, No. 1, of Plate 29 (see Journal, viii., 43).

Exhibits Nos. 2–8 from the water of the Morris and Essex Canal, New Jersey, and all by Stephen Helm.

9. *Melicerta ringens*, living, and in the act of building its tube: by James Walker.

10. *Plumatella* sp.: by James Walker.

11. *Conochilus volvox:* by James Walker.

12. Pollen of Mallow: by Henry C. Bennett.

13. Photomicrograph of statoblasts of *Lophopus crystallinus:* by E. G. Love.

14. Mexican "Jumping Beans," *Sebastiania palmeri* Rose ("Insect Life," iii., 431), or *S. pavoniana* Müll. Arg. (Bull. Torr. Club, xx., 25), showing active motion: by F. D. Skeel.

Mr. Helm remarked that specimens of his exhibit No. 7 had been very scarce in the canal during the present year. They had multiplied in one of his tanks during that time, but had

suddenly died. On the 4th of this month a few specimens were found in the same tank. Also, the exhibit No. 7 showed a peculiarity of this form "No. 1"—twice the usual length, having twice as many processes, with small processes interspersed among the larger ones.

Mr. Walker said that he took his exhibits Nos. 9, 10, and 11 at Glendale, Long Island; and also that he had taken *Bacillaria paradoxa* in abundance in an undoubted fresh-water pool of the tunnel of the Northern Railroad of New Jersey, near Fairview.

Mr. F. W. Leggett stated that he had kept sea water pure in an aquarium for two years.

Mr. William E. Damon stated that he had kept sea water pure in an aquarium, without changing, for fifteen years, by means of floating specimens of *Ulva*.

Prof. Alfred M. Mayer donated specimens of the bark of *Abies balsamea* with capsules of balsam for distribution.

Meeting of October 20th, 1893.

The President, Mr. Charles S. Shultz, in the chair.

Fourteen persons present.

Dr. F. D. Skeel having offered to temporarily store at his residence the exchanges of the Society now being received, and for which there was no shelving now provided at the rooms of the Society, it was resolved that the kind offer of Dr. Skeel be accepted with thanks.

OBJECTS EXHIBITED.

1. Peristome of Moss, *Funaria hygrometrica*.
2. Peristome of Moss, *Mnium undulatum*.
3. Spores and antherozoids of *Chara*.
4. *Podura*, entire insect.
5. Embryo Oysters.
6. Section of fresh-water Pearl from Norway.

Exhibits Nos. 1–6 from the Cabinet of the Society.

7. Sections of cast skin of Chameleon, *Iguana*, showing two principal layers of the same; by L. Riederer.
8. Photomicrographs: Gonococci in leucocytes of human

blood, × 500; Tubercles of tuberculosis in masses attached to walls of human veins; Head of Tapeworm, *Tænia serrata*, showing double row of hooks; Section of bud of Tiger Lily; Transverse section of petiole of *Aspidestris;* Sclerotic cells in pith of stem of *Hoya carnosa;* Transverse section of stem of *Helianthus;* Longitudinal section of stem of Lima Bean: by F. D. SKEEL.

9. Pond-life: by A. D. BALEN.

10. Glass slips with engraved monogram: by H. G. PIFFARD.

Dr. Piffard explained the engraving of his glass slips as accomplished by a loop of platinum wire heated to incandescence by electricity, and used as a pen in writing.

Dr. E. G. Love stated that he marks glass slips by means of a rubber stamp charged with diamond ink—hydrofluorsilicic acid.

MEETING OF NOVEMBER 3D, 1893.

The President, Mr. Charles S. Shultz, in the chair.

Twenty-five persons present.

Messrs. Thomas S. Nedham and Chas. W. Plyer were elected Resident Members of the Society.

The following were appointed by the chair as Committee on Annual Exhibition: Messrs. Henry C. Bennett, Thomas B. Briggs, and L. Riederer.

Dr. Alexis A. Julien read a paper entitled "On Uniformly Stained Cover-Preparations of Micro-organisms free from Distortion." This paper was illustrated by exhibits, as noted in the programme below, and by a demonstration of the method of mounting, and is published in this number of the JOURNAL, p. 1.

The paper was discussed by Mr. Henry C. Bennett and by Drs. H. G. Piffard and F. D. Skeel.

OBJECTS EXHIBITED.

1. Amœboid decorated with a garland of cladothrix.
2. Flagellated vibrios (n. sp.) with stained flagella.
3. Living spirilla of Beggiatoa—the largest known.
4. Amœboid resembling *Difflugia*.

5. Spirillum of Beggiatoa, showing stained flagella.

6. *Arcella*, with pseudopodia projecting.

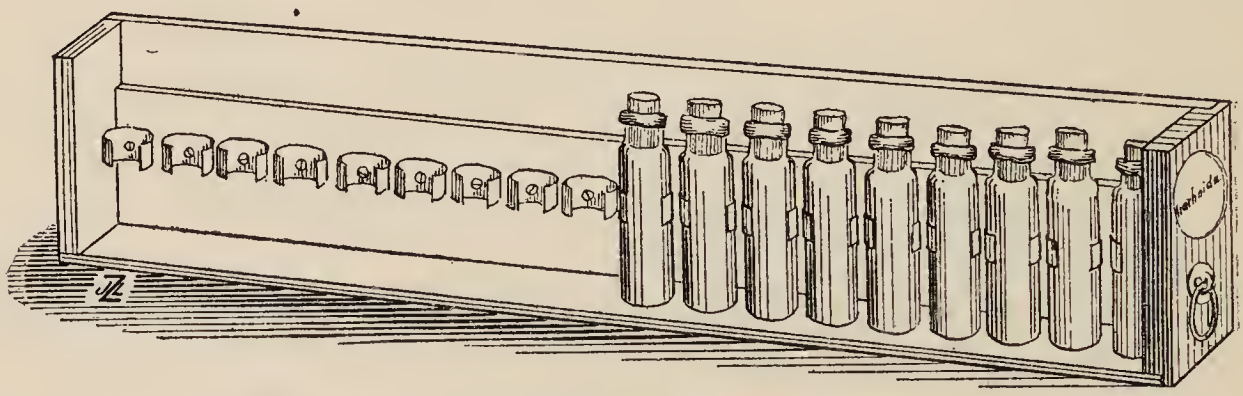
Exhibits 1–6 by A. A. JULIEN.

7. Living *Volvox globator:* by C. S. SHULTZ.

8. Photomicrographs: *Podura*, entire; *Navicula rhomboides*, × 2,000, taken with a lens of extreme under-correction. The same, taken with a Spencer $\frac{1}{10}$, N. A. 1.35: by H. G. PIFFARD.

9. Home-made, convenient trays for bottles containing microscopical specimens: by J. L. ZABRISKIE.

Mr. Zabriskie explained his trays as follows: Four trays of a set are here exhibited, each tray being eleven and one-quarter inches long, one inch wide, and two and one-half inches high, all being intended to stand upright, closely adjoining each other, on the shelves of a cabinet twelve inches in depth



Tray for small bottles.

inside. The bottom and the one side of each tray are made of white wood one-eighth of an inch thick; the rear end of white wood one-quarter of an inch thick; and the front of walnut three-eighths of an inch thick. This thin wood may be purchased of hardware dealers who supply scroll-saw material; the white wood at about four cents a square foot, and the walnut at about twelve cents. The entire inner surface of each tray is pasted with white paper, the reflected light from which allows the easy and rapid examination of an entire set of bottles while in position, by means of a hand lens.

These trays each contain seventeen one-drachm homœopathic bottles. Each bottle is held in place by a brass spring clip clasping the bottle near the middle. The clips are made from soft, thin sheet brass, cut in oblong strips about one-quarter of an inch wide and one and one-quarter inches long, of such size

that, when they are bent round, they enclose about two-thirds of the circumference of a bottle. Each brass strip has a hole punched in the centre, through which is passed a screw, one-quarter of an inch long, for holding the clip in position in the tray. These spring clips hold the bottles with sufficient firmness to prevent the bottles from falling out, and still to allow any one bottle to be extracted and replaced easily without disturbing any of the others.

An additional piece of white wood is inserted behind the bottles, one-eighth of an inch thick, and as long as the long side of the tray, but of such width that it reaches from the bottom of the tray only up to the necks of the bottles. Wood more than one-eighth of an inch thick would appear clumsy for such small trays. This additional piece of wood, which is scarcely noticeable behind the bottles, together with the thickness of the brass, prevents the screws, one-quarter of an inch long, from passing entirely through the wood and projecting at the outer side of the tray. Screws less than one-quarter of an inch long would be too small to handle with convenience.

If larger bottles are needed, larger trays and larger clips must be employed. Some of the specimens exhibited are preserved in dilute alcohol and others in dilute glycerin. But the arrangement is equally convenient for dry specimens in bottles, as diatomaceous material, etc.

An easily interchangeable label is very desirable for such a collection. The method here exhibited can be recommended for ease of construction and satisfaction in use. A slight circular cavity is bored in the face of the front piece of each tray with a centre bit, seven-eighths of an inch in diameter; a disc of white paper is dropped into the cavity; and the paper is retained in position by a single coil of brass wire, which has sufficient spring to resist falling out from any accidental jar, and yet will allow of easy extraction by means of the finger nail, in order to insert another label.

A convenient draw pull, to extract any tray from the cabinet, is made with a three-eighths inch brass screw ring, the screw of which is passed through a small washer, and inserted in the front of the tray below the label.

When all are arranged in a cabinet, any one of a thousand bottles can be quickly selected and extracted.

MEETING OF NOVEMBER 17TH, 1893.

The President, Mr. Charles S. Shultz, in the chair.

Twenty-seven persons present.

The Corresponding Secretary read a paper by Mr. K. M. Cunningham, of Mobile, Alabama, entitled "Notes on some Researches among the Diatomaceæ." This paper is published in the JOURNAL, vol. ix., p. 85.

Mr. Cunningham donated to the cabinet and for distribution dry diatoms stained blue, and a packet of the "moss-like plant" referred to in the paper.

OBJECTS EXHIBITED.

1. Section of foot of human embryo: by HENRY C. BENNETT.

2. Photomicrographs: Gonococcus, × 900; Spirillum of Beggiatoa, × 2,000, showing hyaline membrane surrounding the spirillum: by H. G. PIFFARD.

3. Photomicrograph of group of *Pleurosigma*, by Powell and Lealand's $\frac{1}{16}$ dry objective, supposed to show great diffraction: by F. D. SKEEL.

4. Living *Volvox globator*: by CHARLES S. SHULTZ.

5. Mounted specimens of the same: by CHARLES S. SHULTZ.

6. Circulation in *Nitella*: by A. D. BALEN.

Dr. Piffard donated to the cabinet the photomicrograph of Spirillum of Beggiatoa, and twelve slides prepared by Thum.

Mr. Shultz distributed living specimens of *Volvox* among the members.

MEETING OF DECEMBER 1ST, 1893.

The President, Mr. Charles S. Shultz, in the chair.

Sixteen persons present.

Dr. John A. Fordyce was elected a Resident Member of the Society.

The following Committee on Nominations of Officers was appointed by the chair: F. W. Devoe, William E. Damon, F. D. Skeel, A. Woodward, William G. De Witt.

OBJECTS EXHIBITED.

1. Diatoms from Croton water in November, 40 forms: by H. G. PIFFARD.

2. Living specimens of *Ophryolegna* sp., from human urinary tract: by H. G. PIFFARD.

3. Two photomicrographs of the same: by H. G. PIFFARD.

4. Diatoms from "Queen Silver Polish": by H. G. PIFFARD.

5. *Amphiprora conspicua*, var. *pulchra* Van Heurck, and other forms, from Harlem River: by J. D. HYATT.

6. Diatoms from Honey Meadow Brook, Dutchess Co., N. Y.: by J. D. HYATT.

7. Diatoms from "Silver Polish," under a William Wales $\frac{1}{15}$: by J. D. HYATT.

8. A fruiting fungus on a photographic film: by J. L. ZABRISKIE.

9. Section of lung of dog, injected: by FRANK ABBOTT, JR.

10. Section of kidney of cat, injected: by FRANK ABBOTT, JR.

11. *Spirillum undula:* by FRANK ABBOTT, JR.

12. Anthrax bacillus in liver of rabbit: by FRANK ABBOTT, JR.

Of his exhibit *Ophryolegna*, Dr. Piffard said that these ciliated infusoria had been preserved alive for ten days in distilled water, and were now exhibited in the same medium; they were furnished by Dr. F. Tilden Brown, of this city, who obtained them from the discharges of the urinary passages of one of his patients; that they had been submitted to Dr. Alfred C. Stokes, who determined them as an undescribed species of the genus *Ophryolegna* Ehrb.; and with regard to the photomicrographs of these organisms, that they illustrate the remarks of Dr. Julien at the last meeting respecting the difficulty of mounting delicate organisms without distortion. These photomicrographs show distortion of two kinds—after slow and rapid death.

Dr. Brown, present at the meeting, said that these organisms are found when shreds are cast in pus-laden urine; the shreds come probably from a more remote source than the urethra; the organisms resist the action of slightly acid urine; and that he and Dr. Piffard both saw what they took to be conjugation in some of these organisms.

Mr. Hyatt said of his exhibits of diatoms that this *Amphi-*

prora was common in tide pools of Harlem River; that Dr. Van Heurck gives a figure of the diatom, and the locality as "Harlem River, New York"; that the diatoms of Honey Meadow Brook arise in warm weather as a scum, consisting quite purely of diatoms, one field, as then under the microscope, showing fifty species of the following genera: *Navicula*, *Cyclotella*, *Cymbella*, *Surirella*, *Gomphonema*, *Cocconeis*, *Synedra*, *Achnanthes*, and *Fragilaria*; and also that the "Silver Polish" in question affords multitudes of diatoms, some of them very fine test objects.

Mr. Zabriskie explained his exhibit of fungus as follows: This photograph is a dry-plate lantern slide, one of a set of about one hundred made from negatives by Mr. Leffert Lefferts, taken by him on a trip to Mexico in the fall of 1889. These slides were neatly mounted with mat and cover, and were securely bound with gummed strips around the edges. They were at first frequently used in the lantern, but have been left undisturbed for about one year and one-half, stored in a velvet-lined box on the second floor of the maker's residence. Having occasion to examine them recently, he observed on the film of many of them white, radiating, dendroid patches, one-half of an inch in diameter or less, like some form of crystallization.

On examination with the microscope it was at once seen that each patch was a matured fungus, consisting of white hyphæ, radiating with more or less regularity from a centre, and furnished with numerous branchings, gradually decreasing in size to the attenuated tips. With a magnification of 250 diameters numerous clusters of elliptical, light-brownish spores were found, lying detached upon the film in the neighborhood of the delicate extremities of the branches.

This slide was submitted to Mr. J. B. Ellis, of Newfield, N. J., and from his reply this is probably an undescribed species of fungus. He says it is near *Botrytis reptans*.

Meeting of December 15th, 1893.

The President, Mr. Charles S. Shultz, in the chair.

Twenty-three persons present.

The President announced the death of Dr. Paul Hoffman, a Resident Member of the Society, which event occurred on the 1st inst., and appointed the following committee to formulate action of the Society: Dr. F. D. Skeel, Mr. J. D. Hyatt, and Rev. J. L. Zabriskie.

Mr. William E. Damon, of the Committee on Nominations of Officers, reported for said committee, nominating the present Officers of the Society for re-election to their respective offices in 1894.

OBJECTS EXHIBITED.

1. A simple centrifuge for separating urinary sediment, diatoms, etc.: by H. G. PIFFARD.

2. A Leitz loup, of excellent definition and unusually large field, recently manufactured by Craft on Steinheil's plan: by H. G. PIFFARD.

3. Section of oölitic chert from Dutchess County, New York: by J. D. HYATT.

4. Section of a variety of the same, from the same locality: by J. D. HYATT.

5. Section of oölitic chert from Pennsylvania: by J. D. HYATT.

6. Micrococci in canaliculi of human tooth: by FRANK ABBOTT, JR.

7. Spirillum in canaliculi of human tooth: by FRANK ABBOTT, JR.

8. Transverse and longitudinal sections of the seed of *Phytelephas macrocarpa* Ruiz and Pavon, Ivory nut: by J. L. ZABRISKIE.

9. Transverse section of the seed of *Smilacina racemosa* Desf., False Solomon's Seal: by J. L. ZABRISKIE.

10. The fungus *Æcidium adoxæ*, on the leaf of *Adoxa moschatellina:* by HENRY C. BENNETT.

11. Cyclosis in *Anacharis:* by A. D. BALEN.

12. Circulation in *Daphnia:* by A. D. BALEN.

13. Helices of fine wire for supporting cover-glass preparations: by F. D. SKEEL.

Dr. Frank Abbott explained that the centrifuge exhibited was manufactured by Ernest Leitz, of Wetzlar, Germany, and he demonstrated its action by rotating the instrument for about

one minute, after having inserted a test tube containing urine, and then exhibiting the deposit in the bottom of the test tube.

Mr. J. D. Hyatt remarked on his exhibits that the material belonged to the silurian age, and was formed in shallow seas by the grinding of calcareous matter, acquiring the oölitic form from the concretions of varying colors depositing around a nucleus, and being rolled upon beaches; that in exhibit No. 3 the nuclei are usually minute grains of sand, with the spheres of generally uniform size, and the structure between the spheres like chalcedony; in exhibit No. 4 the nuclei are minute particles of organic matter, with the crystallization radiating from the nuclei, giving the appearance of hornstone; and that the specimen No. 5, from Pennsylvania, has its nuclei of carbonaceous matter with remarkably uniform spheres.

Mr. Bennett explained his exhibit as follows: The slide contains two preparations, opaque and transparent, of the fungus *Æcidium adoxæ*. The opaque preparation shows the peridia in various stages of growth, and the transparent preparation shows a perpendicular section through a burst peridium. The fungus is found in the spring and summer on the under side of the leaf of *Adoxa moschatellina*—Hollow-root—a little, inconspicuous plant, 4 or 5 inches high, growing in woods and moist, shady places in cooler regions. The pale, green flowers have a musky smell, whence its common name, Moschatel.

Dr. Skeel donated for distribution samples of wire helices, of his own construction, for supporting cover-glass preparations.

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L'Académie Royale Suédoise des Sciences de Stockholm: Mémoires, Vol. XVIII., Sections 3, 4 (1893).

L'Académie d'Hippone, Bone: Bulletin (June, 1893).

CORRECTION.—It is due Mr. K. M. Cunningham to state that, in his article, "Notes on some Researches among the Diatomaceæ," published in this JOURNAL, vol. ix., p. 85, by the omission of certain matter contained in his manuscript he is caused to appear as contradicting his own claim of making the first record of "the motility of the protoplasmic covering of the *Amphiprora*." The omission occurs on page 107, immediately preceding the sentence, "One is certain of witnessing a phenomenon that has for many years been of mysterious interest to observers." In this sentence he refers to the cyclosis in *Nitella* and similar organisms, and not to "the activity of the protoplasmic sheath of the *Amphiprora ornata*" of the preceding sentence, as published.—ED.

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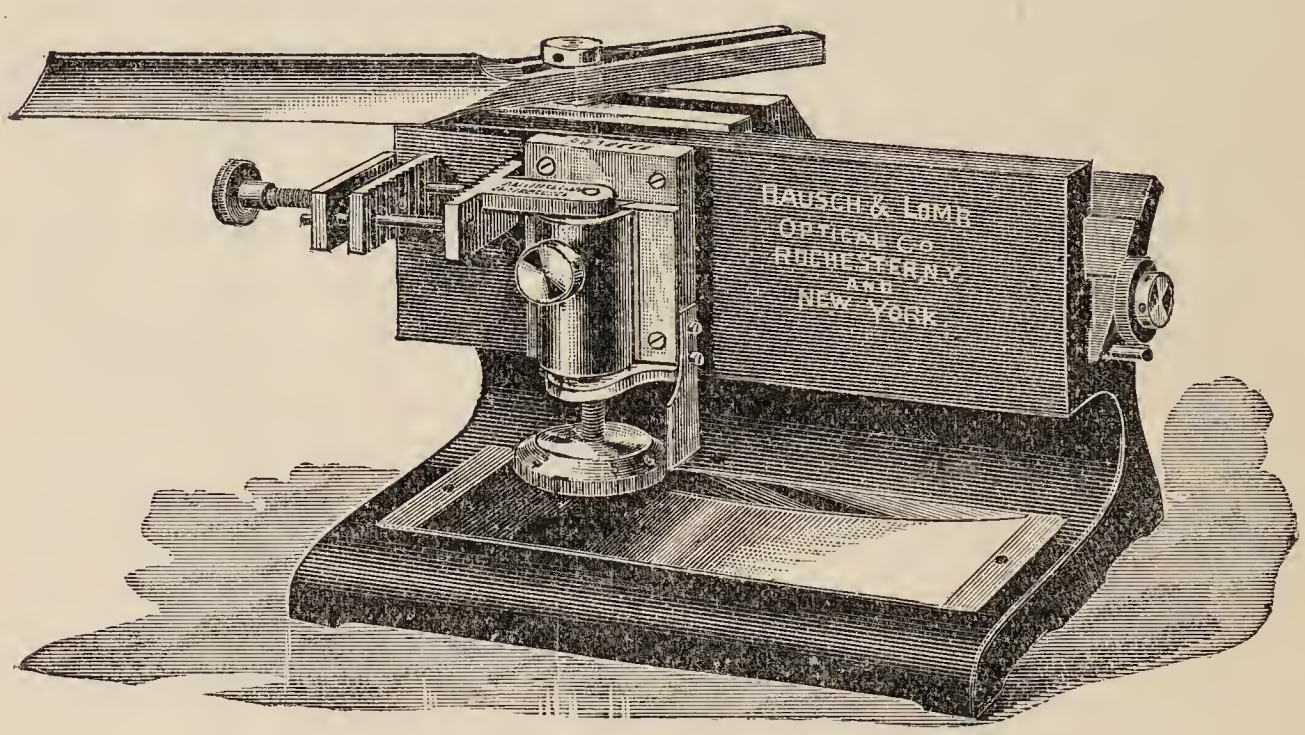

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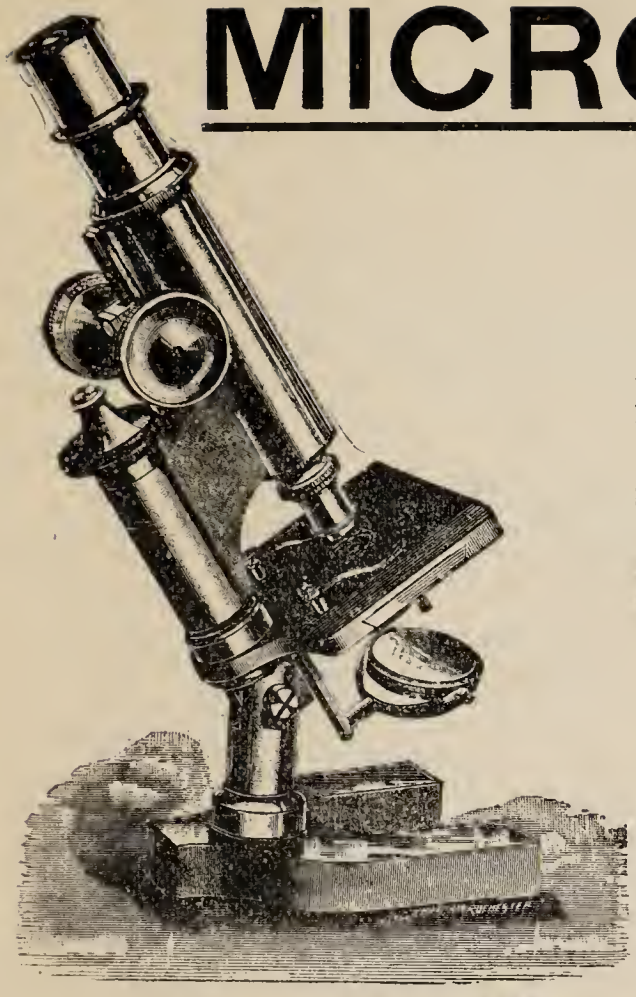

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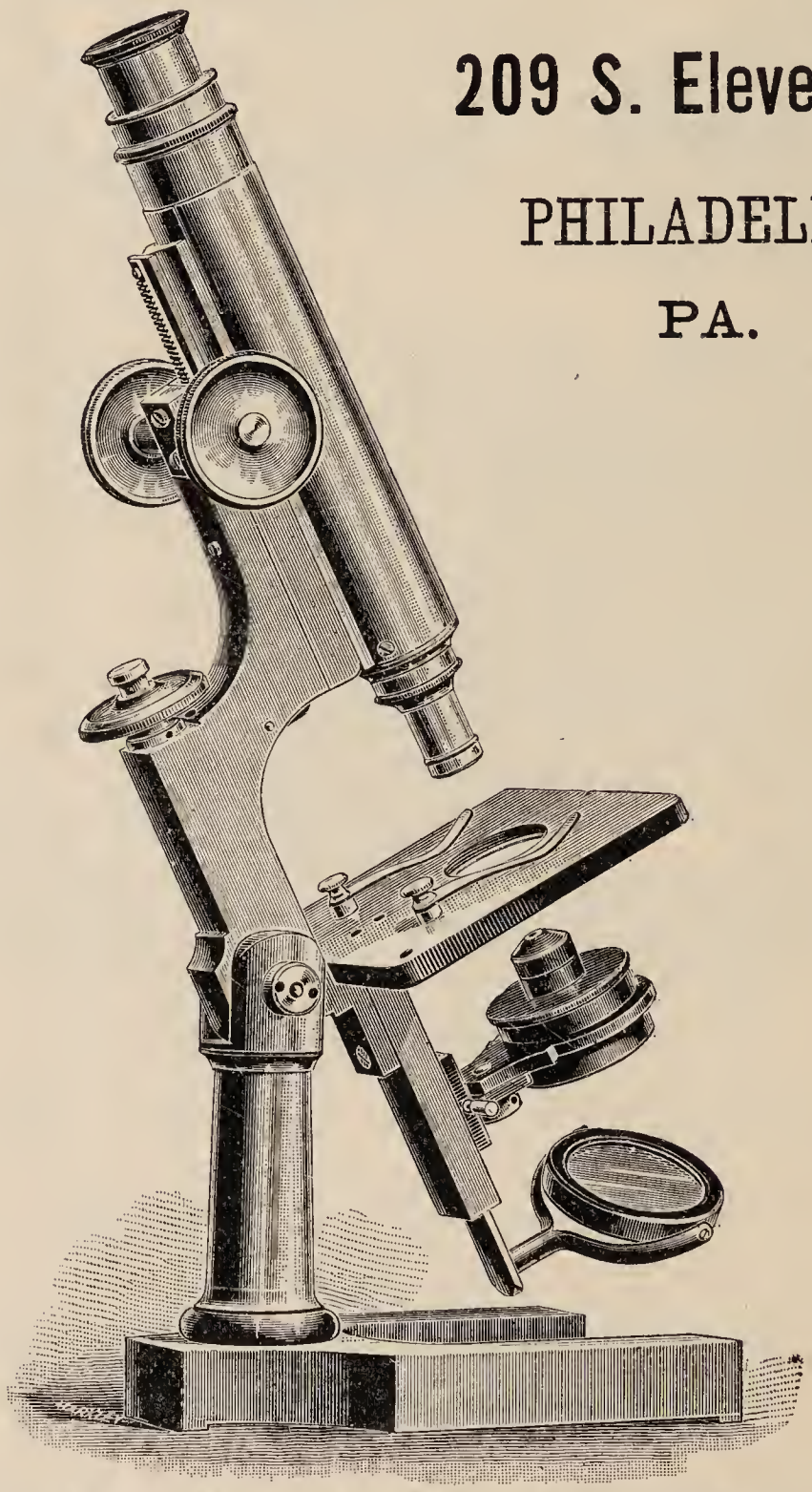

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